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Evaluation of organic and inorganic compounds levels of red wines processed from *Pinot Noir* grapes

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ABSTRACT

Pinot Noir red wines made by malolactic fermentation were studied for studying differences in their chemical profiles with help of a wide spectrum of grape-based and other chemical compounds used in winemaking. Determinations were made with capillary electrophoresis, liquid chromatography, and spectrometry to investigate carbohydrates, organic acids, aldehydes, anthocyanins, phenolic compounds, inorganic anions, and metals. In addition, tot-N, tot-S, and tot-P in the wines were examined.

The wine products showed different profiles of carbohydrates, organic acids, phenolic compounds, and minerals. Especially, saccharose (max. 0.21 g/L), rhamnose (max. 0.45 g/L), fructose (max. 1.9 g/L), and phosphate (max 1.4 g/L) quantities were extremely high in some wines. The results also showed that yeast fermentation in winemaking agitated high production of lactic (max 5.7 g/L) and tartaric (max 1.7 g/L) acids. The red wines processed by cold maceration and natural fermentation gave similar profiles. Only one of the *Pinot Noir* wines entirely differentiated from the others with comparison of carbohydrates and organic acids.

1. Introduction

Wine is much more complex and heterogeneous liquid than any other beverage [1]. Growing grapes, especially crops of *Pinot Noir* grapevine, is demanding to harvest. Due to numerous amount of compounds, which are e.g. water, ethanol, minerals, inorganic compounds, organic acids, carbohydrates, and polyphenol compounds [2], the quality of wine is important. For example, the natural production of wine needs skills for controlling process operations with biochemical and chemical treatments [2,3]. Wine composition correlates with its quality and therefore e.g. grape lignin, grape variety, fermentation with aging, barrel material, and finig chemicals have high impact to the wine products. In addition, aroma and flavour compounds synthetized during fermentation under the influence of winemaking process or in aging with suitable chemicals have significant impact on keeping the wine composition desirable [2–4].

Carbohydrates, organic acids, and sugar alcohols are obtained in fermented plant extracts. Their production can be agitated by external microbes and enzymes, but organic compounds in grapes have a variety roles as being both primary and secondary metabolites. The production of carbohydrates and organic acids is aided by hydrolysis and fermentation with microbes [3,5]. Malic and tartaric acids come from the grapes, whereas lactic, succinic, and acetic acids are originated from the plant after fermentation and maceration processes, [2,6–8]. The fermentation reactions are undertaken by the family of lactic acid bacteria (LAB). On the other hand, the malolactic fermentation (MLF) with *Oenococcus oeni* metabolites controls the LAB [9]. The fermentation often occurs shortly after the end of the primary fermentation, but can sometimes run concurrently with it. *O. oeni*, which metabolizes glucose and produces CO_2, lactic acid, acetic acid, and ethanol. LAB are resistant to low pH and are capable for decomposition of grapes to undesirable by-products in alcohol fermentation, as to acetaldehyde. The bacteria prefer also to metabolize malic acid over sugars but not tartaric acid. The main products of the metabolism are polysaccharides, diacetyl, acetone, acetic acid, and acetaldehyde. The MLF is used to reduce wine acidity by transforming malic acid (dicarboxylic acid) to lactic acid (monocarboxylic acid) [10–12].

Special finig treatments of the grape extract are needed to clarify and stabilize the wine. In addition, in wine processing oak barrels or oak chips are used in order to mature the wine for a specific time. One of the high-tech manipulations of modern winemaking is micro-oxygenation (MOX). It is used to increase the body,

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structure, and fruitfulness of red wines. MOX is based on the use of low levels of oxygen with carefully controlled introduction into the developing wine during an extended period [13]. Likewise, the use of strains of cultured yeast has become an effective resources in winemaking [2].

Usually, determination of the organic compounds in wines has been done with liquid chromatography (HPLC) [14]. However, new techniques like capillary electrophoresis (CE) are used to obtain fast information on ionic compounds and large molecules, such as polymers [15]. Yet, none of the separation techniques have demonstrated for simultaneous determination of organic acids, carbohydrates, and sugar alcohols from wine samples. The methods are used for specific groups of compounds with related structures [3,16]. The advantage of CE over HPLC in wine research is that the same method is suitable for simultaneous separation of sugar alcohols and carbohydrates without manipulation of the original wine extract [17,18]. In that case, special columns, detectors, or derivatization of the compounds are needed to detect the carbohydrates by HPLC [18,6,19]. Regarding to the recoveries, in CE they are very good, as has been demonstrated for tartaric, malic, acetic, succinic, and lactic acids at 98–107% level in the study of 39 bottled Ribeira *Sacra* and *Bierzo* red wines [6]. In general, CE can be validated for determination of metal cations, inorganic anions, organic acids, and carbohydrates, like demonstrated with randomly selected *Pinot Noir* wines [3,16]. According to literature, there are not any publication that tries to demonstrate about simultaneous profiling of organics and inorganics in "good vintage" red wines.

On the other hand, quite often wines are monitored to observe differences in their geographical origin [1,19,20]. Thus, soil minerals and metals have been determined. Furthermore, separate compound groups, like anthocyanins, are studied [1,5,7,10–12]. In spite of controlling of the components in colour, taste, and odour, winemaking processes need increment of chemicals like potassium salts for quality treatment. One of those chemicals is sulphur dioxide, which is added for antioxidative and disinfection purposes [21]. In the vinification processes the use of sulphating agents is essential for improving the quality of wine. Generally, it is measured as the total sulphur (tot-S) in the procedure after nitric acid stabilization with inductively coupled plasma atom emission spectrometry (ICP-AES) [18,22]. Its oxidised species, sulphate and sulphite, can be separated and determined with both HPLC and CE [23]. The permitted level of sulphite in red wines is 160 mg/L. However, for wines containing sugars at higher concentration than 5 g/L, the value is even 300–400 mg/L [24].

The present study describes about profiling of eight *Pinot Noir* red wines produced with MLF processing of grapes. The purpose was to detect differences between them by quantifying both organic and inorganic compounds in the wines that were bottled in the years of 2007–2009. They were selected as representatives of "good vintage" of the young red wines, which have differences in the production. According to our knowledge, the similar study has not carried out previously.

2. Materials and methods

2.1. Materials

Sodium sulphate (Na_2SO_4), triethanolamine, glycolic acid, pyridine-2,3-dicarboxylic acid (2,3-PyDC), 18-crown-6-ether, cyanidin-3-glucoside, syringaldehyde, vanillin, acetaldehyde, and sodium maleate were from Sigma-Aldrich (St. Louis, MO, USA). Tricine, 2-fumaric acid, pyridine, and lactic acid were from BDH (Poole, UK). Ammonium chloride, sodium nitrite, sodium nitrate, sodium hydrogen phosphate ($Na_2HPO_4{\cdot}2H_2O$), sodium chloride, sodium bromide, sodium tetraborate decahydrate, barium hydroxide, sodium carbonate, sodium bicarbonate, and succinic acid were from Fluka (Buchs, Switzerland). Cetyl trimethylammonium bromide (CTAB), urea, citric acid, disodium hydrogen phosphate, and $Ba(OH)_2{\cdot}8H_2O$ were purchased from Merck (Darmstadt, Germany). Formic acid, citric acid, and tartaric acid were from Riedel-de Haën (France). Oxalic acid, malonic acid, acetic acid, tricine and malic acid were obtained from Aldrich (Milwaukee, WI, USA). D-(−)-fructose, D(+)-xylose, D(+)-mannose, D(+)-cellobiose, D-(+)-glucose, saccharose, D-(+)-raffinose, D(−)-mannitol, rhamnose, saccharose, galactose, maltose, arabinose, and ribose were from Sigma-Aldrich (Germany), Fluka Chemie (Buchs, Switzerland), AnalaR Normapur (The Netherlands), Riedel-de Haën (Germany), and Merck (Germany). 1 M NaOH was prepared from Titrisol ampule (Merck Millipore International: Millipore Oy, Espoo, Finland) according to instructions. The ICP-AES standards (Ag, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Na, Ni, P, Pb, S, Sn, Ti, V, Zn, and K) were commercial products. Calcium, phosphate, silver, and sulphur solutions were from ROMIL (ROMIL Ltd, The Source, Waterbeach, Cambridge, UK). Titanium and all other elements not mentioned earlier were from AccuStandard (AccuStandard Europe, Niederbipp, Switzerland). All chemicals were analytical grade and they were used as received. Ultra-pure water (18 MΩ) was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

2.2.1. Liquid chromatography

Inorganic anion analyses were done with an ion chromatograph (IC) with a liquid handling unit, an 818 IC pump, an IC CD detector and an 830 IC interface by Metrohm (Methrom Finland, Espoo, Finland). Data was collected and handled with Methrom IC Net 2.3. The separation column material was polyvinyl alcohol functionalized with quaternary ammonium. The chemical suppression column was a Metrosep A Supp 5 100/4.0 mm. The eluent flow rate was 1.0 mL/min.

2.2.2. Capillary electrophoresis

A P/ACE MDQ capillary electrophoresis instrument (SCIEX Separations, Framingham, MA, USA) equipped with a photodiode array (PDA) detector was used in separation and determination of organic compounds, except aldehydes, which were determined with an Agilent capillary electrophoresis system CE 3D (Hewlett Packard, Walbronn, Germany). Digital electric power supply up to 30 kV, an autosampler (with 100 or 48 vials) and Karate Software (Solid Phase Reversible Immobilization, SPRI) and HP 3D Chemstation data analysis software were employed.

Carboxylic acids, carbohydrates, aldehydes, and ammonium were detected at the wavelengths of 281 nm (indirect detection), 270 nm (direct detection), 192 nm (indirect detection), and 254 nm (indirect detection), respectively. During analyses the temperatures were kept at either 15 °C or 20 ± 1 °C in the MDQ and at 20 ± 2 °C in the Agilent 3D CE instrument by liquid coolant and air conditioning, respectively.

Uncoated fused silica capillaries of 50 μm I.D. and length 50/60 cm (effective length/total length) were used. The samples were injected at the pressure of 0.5 psi (34.5 mbar) in MDQ and at 50 mbar in Agilent CE 3D for 2–10 s. The separation of organic acids and inorganic anions was made with voltages between −9 and −20 kV. In carbohydrate analyses the separation was made in 10–20 kV voltages. Standard calibration and samples were always measured together in the sequence.

Before the CE measurements, new capillaries (from Teknolab, Trollåsen, Norway) were conditioned by rinsing sequentially with 0.1 M HCl, 0.1 M sodium hydroxide, and ultra-pure water. Each solution was used for 20 min and then with electrolyte solution for 20 min. Between analyses, the capillaries were rinsed with 0.1 M HCl and 0.1 M NaOH solution for 3 min and 1 min, and then

with the electrolyte solution for 5 min, respectively. The HCl washing was not used, when the separations were made above pH 8.0 to enable faster stabilization of the capillary surface.

2.2.3. ICP-AES

The ICP-AES instrument (IRIS interpid II XDL, Thermo Fisher Scientific, Vantaa Finland) contained a nebulizer pump with flush pump rate of 130 rpm at 2.40 mL/min and an analysis pump rate of 130 rpm at 2.40 mL/min. The other parameters were RF power 1350 W, nebulizer flow 0.65 lpm and auxilary gas flow 0.50 lpm. The elements were detected at their specific wavelengths.

2.2.4. UV/VIS spectrophotometry

The VIS spectra of anthocyanins were determined with a Varian Cary 1C UV–visible spectrophotometer (Perkin Elmer, Vantaa, Finland). The measurements were done with spectroscopy quality quartz cells at the wavelength of 520 nm. Concentration of the total anthocyanin (C) in the red wine samples were determined by using the equation $C = (A/l\ \varepsilon M_W) * DF$, where A is the absorbance of the wine, l is the path length of the light in the sample (a quartz cuvette of 1 cm), ε is the molar absorptivity (26900 L/mol) of cyanidin-3-glucoside at 520 nm, M_W is the molecular weight of cyanidin-3-glucoside (445 g/mol, the most abundant anthocyanin conjugate found in nature), and DF is the dilution factor (used in measurement of concentrated wines). It is assumed that the samples do not have other components interfering with the measurements at the selected VIS wavelength.

The total phenolic, caffeic, and gallic acid concentrations were measured with UV/VIS spectrophotometry by using absorbance ratios A_{254}/A_{276}, A_{276}/A_{320}, and A_{320}/A_{520}, respectively [25].

2.2.5. Other measurements

Nitrogen (N) in g/kg was measured with the Kjeldahl method documented as ISO standards [26,27]. Nitrogen was also measured with Dumas modified method [28]. The pH measurements were carried out using a Denver model 20 pH metre with combination electrode (Denver Instrument Company, Denver, CO, USA). The combination electrode was calibrated with pH 4.00 (±0.01), 7.00 (±0.01), and 10.00 (±0.01) with commercial buffers (Merck, Darmstadt, Germany). The total amount of organic compounds (TOC) were measured as TC and TOC measures that were accomplished using a Shimadzu TOC analyser (TOC-L, Vantaa, Finland). The instrument provides a concentration range of 4 μg/L to 30 g/L. TOC analyser uses a 680 °C temperature and the combustion with ozone for catalytic oxidation.

2.3. Preparation of solutions

2.3.1. Carboxylic acids

Organic acids were analysed in an electrolyte solution that consisted of 20 mM 2,3-PyDC, 30 mM tricine, 2 mM $Ba(OH)_2$, 0.5 mM CTAB, and 2 M urea. The pH of the electrolyte was adjusted to 8.06 with triethanolamine [16]. The stock solutions of each organic acid were prepared in the concentration of 10 g/L in ultra-pure water. The working standard solutions with a concentration range from 1 mg/L to 150 mg/L. They were made by appropriate dilutions from the stock solutions with ultra-pure water. The stock solutions were stored at +4 °C. The limit of detection (LOD, signal-to-noise ratio (S/N) of 3) was determined with standards diluted in ultra-pure water, while the limit of quantification (LOQ S/N of 10) was determined in the red wine matrices, for all the compounds.

2.3.2. Mono and dicarbohydrates, sugar acids

The electrolyte solution contained 130 mM NaOH and 36 mM Na_2HPO_4. It was prepared by mixing 450 mM stock solution of $Na_2HPO_4{\cdot}2H_2O$ with 1 M sodium hydroxide solution. The electrolyte solution has pH of 12.6 [3]. For calibration the mixtures of carbohydrates and sugar acids were prepared from stock standard solutions by dilution with ultra-pure water. Concentrations of 1, 2, 3, 4, 5, 10, 15, 20, and 25 mg/L were used for calibration.

2.3.3. Aldehydes

Acetaldehyde, vanillin, and syringaldehyde were prepared for working solutions as described for carboxylic acids. They were separated in aqueous sodium tetraborate solution at pH 9.3 [7,24,29].

2.3.4. Ammonium

Ammonium was prepared from 1 g/L ammonium chloride solution by diluting the stock solution to the working concentrations. Ammonium was analysed in the method developed for inorganic cations described elsewhere [30,31]. The electrolyte solution contained 9 mM pyridine, 12 mM glycolic acid, and 5 mM 18-crown-6 ether at pH 3.6. The pH was adjusted with 0.1 M HCl.

2.3.5. Inorganic anions

The stock solution for the IC eluent was 0.5 M Na_2CO_3–0.5 M $NaHCO_3$ containing acetonitrile as a preservative. The mobile phase in IC measurement of anions was either 4.8 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ or separate solutions of 0.5 mM Na_2CO_3 and 0.5 mM $NaHCO_3$. The solutions were needed to separate phosphate and sulphate in wine samples. Calibration sulphate in wine samples in wine samples. Calibration standards of F^-, Cl^- NO_2 Br, NO_3, HPO_4, and SO_4 in various concentration compositions were prepared from the sodium salts of the inorganic anions into ultra-pure water. The concentration range was from 0.2 mg/L to 50 mg/L for each. The limits of detection were measured with a standard mixture containing F^-, Cl^-, NO_2, Br, NO_3, PO_4, and SO_4 at 0.2, 0.5, 0.2, 0.5, 0.5, 0.5, and 0.2 mg/L concentrations, respectively.

2.3.6. Metals

The calibration standards were prepared from a multi-elemental standard mixture in 0.5% nitric acid-water solution. The concentration range for the studied 21 elements (Ag, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Na, Ni, P, Pb, S, Sn, Ti, V, Zn, and K) was from 1 μg/L to 100 mg/L. The stock solutions for calcium, phosphate, silver, and sulphur were 1.0 g/L. Titanium and the rest of the metals were prepared from the stock solutions of 100 mg/L.

2.4. Wine samples

The red wine samples were purchased directly from the manufacturers or with off-licenses to Finland. *Pinot Noir* was the grape of all samples. According to universal legislation in wine manufacturing 75–85% of the grapes must be from the same district or at 75% level (min) the cultivation year of the grapes must be the same.

All the studied wines were made by malolactic fermentation process (MLF). The bottles were opened for the sensory evaluation [3] and after dosing immediately closed tightly with the cap. The rest of the wines were divided into three portions when transferring to amber bottles in order to protect against sunlight and air. They were stored at 4 °C. Each of the wine portions was analysed six times with two replicates. The background of the red wines in our study are listed in Table 1. The winemaking processes were natural fermentation (Wine 1), fermentation without details (Wine 2, Wine 4), biodynamic fermentation (biodynamic = wine produced from organically grown grapes, Wine 3), micro-oxygenation (Wine 5), yeast fermentation (Wine 6), wild fermentation (Wine 7), and cold fermentation (Wine 8).

In most of the analyses (TOC, total-N, total-P and total-S, anthocyanins, phenols) the wines 1–8 were used as received. In CE the

Table 1
The *Pinot Noir* red wine samples in the study. The wines have been provided directly from the manufacturers or from the off-license in Finland.

Wines	Alcohol (%)	Other information	Country and Year	Extract conc. Trade description	pH measured in the study	Total Acids, trade description [g/L]
Wine 1 Producer: Jackson, Wine Estate, Name La Crema, Los Casnecos AVA *Processed by natural fermentation*	14.5	Handpicked Destemmed 5 days cold maceration Appellation: 95% Los Carneros, 5% Sonoma CoastComposition: 100% Pinot NoirClonal Selection: 115, 667, 777, 828, Calera, 2AType of Oak: 100% French, 35% new barrels,medium and medium plus toast levelsTime in Barrel: 10 months http://www.lacrema.com/assets/client/file/2007-LosCarnerosPinotNoir.pdf	U.S.A. 2007	0.57 g/ 100 mL (pH 3.63)	3.72	5.7
Wine 2 Producer: Hahn Family WinesName: Cycles GladiatorRegion California *Processed by malolactic fermentation*	13.9	http://store.hahnfamilywines.com/Cycles_Gladiator_Pinot_Noir_California_2008	U.S.A. California 2008	5.80 g/L (pH 3.65)	3.77	5.9
Wine 3 Le Ban Saint-Aubin *Biodynamic processing*	12.5	No SO_2 during winemaking process, a small amount at bottling for stabilization	France 2007	(pH 3.7)	4.02	5.3
Wine 4 Juliches 99 Rows PN *Unknown wine making*	14.5	Unknown	New Zealand 2009	25 g/L	3.85	5.3
Wine 5 Producer ErathName Erath Oregon Pinot Noir, Region Oregon *Processed by micro-oxygenation*	13.5	Cross Flow Filtration Micro-oxygenation stainless steel, oak chips	U.S.A. Oregon 2007	32 g/L	3.85	5.92
Wine 6 Matua Valley Malborough PN[6]) *Processed by malolactic fermentation and yeast addition*	14	Malolactic fermentation, yeast	New Zealand 2009	30 mg/L	3.63	6
Wine 7 Producer Viña ErrazurizName Errázuriz Wild Ferment Pinot NoirRegion Casablanca Valley *Processed by malolactic fermentation and yeast*	14	Composition: 100% *Pinot Noir* Appellation: Casablanca Valley 3-6 days cold maceration destemmed Indigenous native yeasts 9 months in Burgundy-style French oak barrels, 50% new http://www.errazuriz.com/errazuriz/english/pdf/PNWF08e.pdf	Chile 2008	30 mg/L (pH 3.54)	3.51	6.02 Residual Sugar: 2.28 g
Wine 8 Producer Viña LeydaName Leyda Las Brisas Pinot NoirRegion Leyda Valley *Processed by cold maceration and natural fermentation*	14	Handpicked, destemmed cold maceration + 30% whole clusters to semi-carbonic maceration selected yeast inoculation used French oak 10 months	Chile 2009	0.57 g/ 100 mL (pH 3.63)	3.67	Residual sugar 3.3

anions of carboxylic acids were analyzed from the wines after dilution to 1:20 and 1:50 (v/v) with 20 mM NaOH solution. For IC and spectrophotometric analyses, which needed dilution with ultra-pure water. For IC measurements the wines were diluted for measurements of Cl^-, HPO_4^{3-}, and SO_4 (for chloride 6 mL wine add. 12 mL aq. or 5 mL wine add. 15 mL aq.; in phosphate and sulphate analyses 4 mL wine add. 50 mL aq., 1 mL wine ad. 20 mL aq., and 14.5 mL wine ad. 14.5 mL aq.). When the sulphate concentration was out of the concentration linearity, the wines were still more diluted to ratios of 0.5:25 and 0.25:14.75 (v/v). Concentration calibration of the inorganic ions was made with 5, 10, 20, 40, and 60 mg/L solutions, containing all the seven inorganic anions. The wine samples were treated for metal analyses with nitric acid. In Vis–spectrophotometric determination the wines were diluted in ultra-pure water (2 mL wine add. 8 mL aq.) and measured without filtration.

3. Results and discussion

3.1. The red wines

The results showed differences in the profiles of the eight studied red wine (Wine 1-Wine 8, Table 1). However, significant variations based on the total quantities of organics (tot-Org) and minerals (tot-Inorg) could not be recognized (Table 2). Nevertheless, the lowest composition of tot-Org were in the Wines 1, 3, and 5 representing natural, biodynamic, and micro-oxygenation fermentation, respectively. The highest one was in Wine 6 (yeast fermented). On the contrary, the wines 4 and 6–8 had the lowest tot-Inorg. Then, when the individual organic and inorganic compounds were closely studied, there were remarkable differences between the profiles: Biodynamically produced grapes fermentation without SO_2 and micro-oxygenation treated grapes (Wines 3 and 5, respectively) gave the lowest organics contents. Furthermore, the Wines 4 and 6 from New Zealand contained very low mineral levels.

3.2. Determination of carboxylic acids

Totally 19 organic acids and sugar acids were studied in the red wines (Fig. 1). The most dominant compounds were carboxylic acids, independently from the procedures used in winemaking. The highest total quantity of organic acids were observed in Wines 2 and 8. Separation of the acids gave the highest quantities to lactic, acetic, succinic, and tartaric acids (Table 2). The electropherograms in capillary electrophoresis measurements showed that there were a couple of specialities among the studied samples. One of them was Wine 4 (MLF without details), which did not

contain formate and maleate at all. However, the most unequal sample was Wine 6, because it was processed in the presence of yeast, but the extract did not contain maleate and succinate. Instead, Wine 6 had the highest amounts of acetic, malic, and lactic acids as well as acetaldehyde (see section 3.4).

The result was surprising, since succinic acid is commonly found in wine and in the fluids of ripened grapes [2,8]. Furthermore, because Wine 6 contained the highest alcohol percentage (Table 2), we monitored the carbohydrate amount that was very low in comparison with the other *Pinot Noir* wines. Therefore, it was assumed that the most acidic wine in the study was Wine 6 (Figs. 2A and 2B). Although, sweetness and acidity of the wines is based on sugar and organic acid concentrations, respectively, the correlation of the acidity was studied with the commonly acceptable equation for Treatable Acidity (TA), which is determined with quantities of malic and tartaric acids. Then, the TA/tartaric acid ratio was calculated with the total organic acid concentration (tartaric acid and malic acid) divided by tartaric acid concentration. The acidity was also evaluated from sulphuric acid formation. Then, the value is measured from TA concentration divided by sulphate concentration in the wine.

Noteworthy is that the two acidity values cannot be compared, because they are calculated from different starting values (Fig. 2A). Based on the results, the wines could be classified by their sugar and acid quantities (Fig. 2B). The results verified that Wine 6 was abnormal due to the low tartaric acid amount. As a comparison, it was the highest in Wine 3 (2.2 g/L) and moderately high in the other wines (1.4–1.8 g/L). Because of that it could be suggested without background knowledge that Wine 6 was processed without potassium tartrate addition (Fig. 3).

Overall, that result was unexpected because tartrate is a component from grapes. As noticed in acidity tests with capillary electrophoresis, tartrate concentrations are very high and may activate tartrate in crystallization with calcium but also with other earth-alkali metals [14,20]. Another unpredictable result was that tartrate quantity was high in biodynamically cultivated grapes. Thus, the wines 1–3 and 5 had the highest potassium measures. In these wines therefore, both tartrate and sulphate (bisulphite, hydrogen sulphite oxidation to sulphate during the process) influenced on calculation of wine acidity.

The highest acidity were in Wines 2 and 8 (Table 2). Malic and formic acids were found only in minor quantities. But, it was interesting that Wine 4 did not have formic acid at all. Profiles of organic acids showed evidence on possible transformation or conversion of malic acid to lactic acid, which was detected from high lactic acid concentration (Fig. 1). It was also noticed that only in Wines 1, 4, 5, and 7 the acidity was not changed during storaging. The second metabolic acid, acetic acid, was in some wines above 1 g/L, which means that the composition was above the recommendations [7]. However, during the course of winemaking and in the finished wines acetic, butyric, lactic, and succinic acids have a significant role in the quality of the products. However, generally high concentration of acetic acid is an exception [32], because the allowed limit of volatile acids is 1.08 g/L and 1.2 g/L in white and red wines, respectively. From the results obtained it can be concluded that in Wine 2 the fermentation process was not accomplished before bottling. It was supported by calculations with the concentration ratio between malic acid and lactic acid, which was 0.32 in Wine 2. In the other wines the values were only 0.04–0.08. In Wines 4 and 6 malic acid was not observed at all.

3.3. Determination of carbohydrates

Winemaking process results in grape juices containing glucose and fructose, which are for production of alcohol [33,34]. According to our study, glucose and fructose were at quite low concentrations in the *Pinot Noir* wines, except in the wines 4 (MLF, details not informed) and 5 (MOX), where the fructose amounts were extremely high (Fig. 4). On the contrary, according to our results the bottled wines had low amounts of galactose, although it originates from the grape hemicelluloses. That means that the processes may not degrade hemicelluloses to galactose or then the fermentation synthesized it to lactic acid. In most wines, there was a little saccharose, which is not a natural constituent of grapes, but may be added to the wines for the purpose of capitalisation. Saccharose ensures ethanol production during alcohol fermentation, if the source materials are spent [34]. In general, when the basic carbohydrates are low in a wine, saccharose is added for the source material of glucose and fructose.

Because of the high carbohydrate concentration in Wine 5 (MOX), it was assumed that it was the sweetest of the studied *Pinot Noir* wines (see also Figs. 2A and 2B, Table 1). Wines 6–8 (processed with yeast, wild, and cold fermentation, resp.) contained high galactose content. It is known, processed galactose, fructose, and xylose may vary during wine aging, when wine is manufactured in oak wood barrels [29,34,35]. Especially, when grape skins are macerated to grape juice, saccharides are produced from polyphenols in a long aging process. In addition, fermentation of hemicelluloses also release saccharides in barrels [34,36]. According to that in our study, too, the wines 4 and 5 have extremely high amounts of fructose, 0.7 g/L and 1.9 g/L, respectively, of which the latter process contains oak wood chips as documented in the wine informative label. Noteworthy is that the total saccharide amounts of Wines 4 an 5 were 1.4 g/L and 2.5 g/L, respectively, when the general quantity was below 0.8 g/L. As to carbohydrates, the sweetest wines were therefore Wine 4 and Wine 5. In addition, Wine 1 and Wine 2 contained quite high total carbohydrate quantities because of the high rhamnose and xylose amounts.

3.4. Anthocyanins, phenolic compounds, and aldehydes in the wines

Low temperature maceration (5–15 °C) prior to fermentation is designed to improve the extraction of grape-based compounds (anthocyanins, phenolol and phenolic acids) to the wine [37]. Our samples (Wines 1 and 8), have also gone thorough the cold maceration. The impact of cold-maceration before fermentation is thought to improve colour in young red wines. The success of combination technique is dependent on time, temperatures and e.g. sulphur dioxide levels [8,11,37]. Aromatic aldehydes are origin from lignin and they are produced during MLF. The absorbance of the red wine at 520 nm wavelength is useful for measuring the total pigment amount. The efficiency of the combination method is noticed from the anthocyanin concentrations of Wines 1 and 8 (Table 2). Furthermore, Wine 3 which was made from biodynamically cultivated grapes, contained anthocyanins more than on average. Therefore it was assumed that bioprocessing has a positive effect on the extraction of the pigments into the grape fluids. On the contrary, in the presence of yeast (Wine 6) the production of anthocyanins was reduced. The similar effect was noticed in the published study on the fluids made of traditional Portuguese, Saint Laurent, and Blaufrankisch grapes during maceration [34]. It was surprising that the new wine processing method, micro-oxygenation (MOX), produced only average amounts of anthocyanins (Wine 5). Most probably, the reason was oxidation of some anthocyanins, which lose their visible absorption in oxidation, and absorb at lower wavelength due to functional changes in the structures [35,37,38] and formation of polymers (e.g. dimers) [39]. Carbohydrates that are usually bound to anthocyanins are glucose, galactose, rhamnose, and arabinose.

In our study we measured concentrations for caffeic acid, gallic acid, and total phenols, which were calculated with the absorbance ratios of A254/A276, A276/A320, and A320/A520 [25]. The

Table 2
Concentrations of the studied compounds in red wines processed from *Pinot Noir* grapes.

Pinot Noir Red Wine	Wine 1 C_{ave} (mg/L)	Wine 2 C_{ave} (mg/L)	Wine 3 C_{ave} (mg/L)	Wine 4 C_{ave} (mg/L)	Wine 5 C_{ave} (mg/L)	Wine 6 C_{ave} (mg/L)	Wine 7 C_{ave} (mg/L)	Wine 8 C_{ave} (mg/L)	Comments
Alcohol (%)	14.5	13.9	12.5	14.5	13.5	14.0	14.0	14.0	Trade description
Acidity									
pH	3.63	3.65	3.7	Not mentioned	Not mentioned	Not mentioned	3.54	Not mentioned	Trade description
pH (at 20 °C)	3.72 ± 0.10	3.77 ± 0.10	4.02 ± 0.10	3.85 ± 0.10	3.85 ± 0.10	3.63 ± 0.12	3.51 ± 0.11	3.67 ± 0.13	Measured in the work pHs should be 3.1–3.7
ΣOrganic acids before bottling (g/L)	5.7	5.9	5.3	5.3	5.92	6.0	6.02	Not mentioned	Trade description
TA/Tartaric Acid	0.185 ± 0.02	0.182 ± 0.003	0.219 ± 0.001	0.144 ± 0.003	0.188 ± 0.004	0.001 ± 0.011	0.172 ± 0.001	0.162 ± 0.013	
TA/Sulphate	0.041 ± 0.001	0.054 ± 0.001	0.022 ± 0.000	0.021 ± 0.000	0.035 ± 0.001	0.024 ± 0.000	0.041 ± 0.001	0.021 ± 0.001	
ΣOrganic acids in bottled wine average, (g/L)	5.83	13.6	6.8	6.32	6.18	8.71	5.94	12.8	
Sweetness									
∑Monosaccharides average, (mg/L)	697 ± 12	740 ± 3	439 ± 4	1385 ± 9	2499 ± 2	275 ± 2	453 ± 5	270 ± 2	
∑Disaccharides and sugar acids, average (mg/L)	450	1118	1453	3000	1357	703	579	835	
Residual sugar (g)	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	2.28	3.3	Trade description
Total organics tot-Org, average (g/L)	21.1	24.78	21.68	26.14	20.82	28.16	26.54	27.22	
Total quantities									
Total inorganics tot-Inorg, average (mg/L)	1406	1295	1274	1075	1228	1078	1152	1124	
Tot-N (mg/L)	20.5 ± 0.5	50.5 ± 0.3	24.0 ± 0.5	46.0 ± 0.6	19.1 ± 0.7	38.0 ± 0.2	72.5 ± 0.9	51.0 ± 0.4	
Tot-S (mg/L)	121.4 ± 1.57	190.5 ± 3.00	131.2 ± 8.66	164.4 ± 5.701	99.1 ± 3.503	116.7 ± 4.222	251.7 ± 4.944	196.5 ± 4,539	
Tot-P (mg/L)	250.7 ± 2.4	240.4 ± 12.93	181.7 ± 2.94	164.4 ± 3.194	174.5 ± 3.31	119.5 ± 4.844	252.6 ± 4.5611	388.0 ± 10.49	
SO_2 Calculated in the study (mg/L)	81.2 (CM*)	127.4	87.8 (BIO)	109.9	66.3 (MOX)	78.0	168.3 (CM*)	131.4 (CM*)	
Saccharides (mg/L)									
Saccharose	78.1 ± 1.0	126 ± 0.6	71.3 ± 1.2	213 ± 1.0	189 ± 1.0	45.6 ± 1.0	43.8 ± 1.0	52.6 ± 1.2	$y = 509.44x - 3633.2$ $R^2 = 0.9995$
Galactose	39.6 ± 1.7	41 ± 1.0	17.9 ± 0.2	62.1 ± 10	31.8 ± 2.5	128.4 ± 2.3	93.7 ± 3.1	112 ± 1.5	$y = 590.7x - 1325.2$ $R^2 = 0.9986$
Fructose	29 ± 2.5	50 ± 1.5	83 ± 1.0	720 ± 14	1892 ± 50	10 ± 1.5	21.5 ± 1.5	11.7 ± 1.6	$y = 254.91x + 1187.5$ $R^2 = 0.9989$
Glucose	50.9 ± 2.9	40 ± 1.7	10 ± 1.6	50.4 ± 1.7	49.5 ± 2.9	10.5 ± 1.2	15.6 ± 1.0	16.2 ± 1.2	$y = 473.66x - 242.4$ $R^2 = 0.9983$
Rhamnose	262 ± 28	452 ± 2.0	124 ± 7.5	287 ± 21	201 ± 5.0	31.7 ± 2.5	245 ± 20	36 ± 2.3	$y = 245.16x - 219.8$ $R^2 = 0.9987$
Xylose	237 ± 31	28 ± 5.0	133 ± 10	52 ± 4.7	135.4 ± 6.5	48.4 ± 4.3	33 ± 5.2	41.9 ± 4.5	$y = 401.83x - 1657.3$ $R^2 = 0.9991$
Organic acids (mg/L)									
Formic acid	123 ± 0.4	159 ± 0.2	142 ± 0.3	10 ± 0.04	96 ± 0.8	86 ± 0.60	201 ± 1.0	108 ± 0.6	$y = 490.11x - 136.1$ $R^2 = 0.9998$
Succinic acid	637 ± 0.2	801 ± 0.3	689 ± 0.2	853 ± 0.1	624 ± 0.5	10 ± 0.02	534 ± 0.7	807 ± 0.5	$y = 337.37x - 129.01$ $R^2 = 0.9999$
Malic acid	172 ± 0.2	447 ± 0.4	146 ± 0.1	10 ± 0.02	174 ± 0.2	1614 ± 1.5	144 ± 0.2	101 ± 0.2	$y = 103.42x + 44.313$ $R^2 = 0.999$
Tartaric acid	1833 ± 2.7	1810 ± 1.6	2177 ± 2.0	1427 ± 1.8	1869 ± 2.2	10 ± 0.01	1711 ± 2.0	1677 ± 1.6	$y = 104.78x - 142.44$ $R^2 = 0.9999$
Acetic acid	792 ± 0.5	841 ± 0.5	605 ± 0.5	674 ± 0.4	1001 ± 1.1	1297 ± 1.5	741 ± 0.5	708 ± 0.1	$y = 458.97x - 10.286R^2 = 0.9995$
Lactic acid	2274 ± 2.0	1382 ± 1.1	3039 ± 2.0	3362 ± 1.5	2414 ± 1.5	5710 ± 2.7	2606 ± 2.1	2261 ± 2.3	$y = 1710.7x + 611.88$ $R^2 = 0.9988$
Oxalic acid	<2	<2	<2	<2	<2	<2	<2	<2	$y = 124.66x + 9.3953R^2 = 0.999$
Citric acid	<2	<2	<2	<2	<2	<2	<2	<2	$y = 99.225x - 715.23$ $R^2 = 0.9969$
2-furoic acid	<2	<2	<2	<2	<2	<2	<2	<2	Not quantified
Anthocyanins (mg/L)	53.2 ± 0.053	42.5 ± 0.021	40.1 ± 0.112	41.8 ± 0.029	29.0 ± 0.081	23.3 ± 0.54	46.8 ± 0.09	54.4 ± 0.22	rsd 0.02–0.28%
Aldehydes									
syringaldehyde	2.4 ± 0.1	nd	2.3 ± 0.1	nd	1.3 ± 0.0	1.5 ± 0.1	3.1 ± 0.0	0.8 ± 0.0	rsd 0.1–0.4%
vanillin	5.2 ± 0.01	nd	1.0 ± 0.01	nd	3.7 ± 0.01	3.9 ± 0.01	3.7 ± 0.01	1.8 ± 0.00	rsd 0.1–0.5%
acetaldehyde	10.1 ± 0.01	30.0 ± 0.10	7.0 ± 0.10	29.0 ± 0.01	9.1 ± 0.05	18.9 ± 0.07	10.2 ± 0.01	20.0 ± 0.04	$y = 0.2074x + 1.1554$ $R^2 = 0.9915$

(*continued on next page*)

Table 2 (*continued*)

Pinot Noir Red Wine	Wine 1 C_{ave} (mg/L)	Wine 2 C_{ave} (mg/L)	Wine 3 C_{ave} (mg/L)	Wine 4 C_{ave} (mg/L)	Wine 5 C_{ave} (mg/L)	Wine 6 C_{ave} (mg/L)	Wine 7 C_{ave} (mg/L)	Wine 8 C_{ave} (mg/L)	Comments
Caffeic acid, Gallic acids, Total phenol content									
A_{254}/A_{276}	1.1226 ± 0.001	1.1126 ± 0.004	1.1292 ± 0.005	1.0284 ± 0.004	1.065 ± 0.001	1.0478 ± 0.001	1.542 ± 0.002	1.0268 ± 0.003	
A_{276}/A_{320}	1.0773 ± 0.002	1.0898 ± 0.003	1.1161 ± 0.001	1.0634 ± 0.001	1.0957 ± 0.002	1.0504 ± 0.001	1.0566 ± 0.002	1.110 ± 0.002	
A_{320}/A_{520}	1.0999 ± 0.001	1.3378 ± 0.001	1.6237 ± 0.001	1.3926 ± 0.001	2.1426 ± 0.002	2.5625 ± 0.002	1.3123 ± 0.003	1.1824 ± 0.002	
Metals/Ammonium (mg/L)									
K^+	1077 ± 10.1	1006 ± 20.3	1013 ± 16.01	740 ± 10.88	930 ± 9.08	795 ± 10.23	857 ± 13.09	834 ± 11.06	y = 0.3085x + 0.0103 R^2 = 0.996
Na^+	181 ± 20.0	158 ± 14.6	144 ± 12.0	165 ± 6.0	151 ± 12.5	113 ± 8.58	103 ± 13.9	99.5 ± 4.65	y=0.3603x + 0.0097 R^2 = 0.9975
Ca^{2+}	34.25 ± 2.584	43.6 ± 3.9322	44.6 ± 1.5737	56.9 ± 4.587	56.3 ± 4.178	47.0 ± 4.056	59.7 ± 4.663	55.5 ± 2.0998	y = 0.2269x + 0.1363 R^2 = 0.9774
Mn^{2+}	1.197 ± 0.0561	1.406 ± 0.0508	0.741 ± 0.0108	0.697 ± 0.0236	1.456 ± 0.0454	1.665 ± 0.08995	1.194 ± 0,04398	2.256 ± 0.1227	y = 0.1638x − 0.001 R^2 = 0.9965
Li^+	0.032 ± 0.004	0.032 ± 0.003	0.002 ± 0.001	0.003 ± 0.001	0.009 ± 0.001	0.008 ± 0.001	0.004 ± 0.001	0.008 ± 0.001	y = 0.059x + 0.0003 R^2 = 0.9997
Ba^{2+}	0.346 ± 0.0148	0.280 ± 0.00412	0.032 ± 0.00217	0.155 ± 0.00421	0.439 ± 0.006	0.181 ± 0.0049	0.180 ± 0,00339	0.175 ± 0,0061	y = 0.0245x + 0.0031 R^2 = 0.9889
Mg^{2+}	109.783 ± 4.233	84.5355 ± 2,841	69.1654 ± 3.450	109.1667 ± 2.5167	89.701 ± 1.796	121.2499 ± 5.199	122.5998 ± 3.6499	127.9667 ± 3.0166	y = 1.0821x + 0.0014 R^2 = 0.9976
Sn^{2+}	0.562 ± 0.034	0.623 ± 0.051	0.688 ± 0.018	0.607 ± 0.009	0.659 ± 0.013	0.652 ± 0.018	0.680 ± 0.016	0.680 ± 0.010	y = 0.059x + 0.0003 R^2 = 0.9997
Fe^{3+}	0.925 ± 0.0493	1.845 ± 0.0720	1.822 ± 0.115	0.517 ± 0.0229	1.355 ± 0.1325	0.992 ± 0.0854	2.127 ± 0.17533	2.210 ± 0.2411	y=0.0375x − 0.0039 R^2 = 0.9566
Zn^{2+}	0.519 ± 0.05499	0.498 ± 0.0166	0.315 ± 0.00968	0.330 ± 0.0649	0.357 ± 0.01823	0.535 ± 0.02769	0.621 ± 0.01935	0.323 ± 0.02149	y = 0.2387x + 0.0021 R^2 = 0.9998
Cu^{2+}	0.025 ± 0.0011	0.056 ± 0.0064	0.067 ± 0.00559	0.013 ± 0.00478	0.017 ± 0.00749	0.024 ± 0,00476	0.039 ± 0,00692	0.027 ± 0.0043	y = 0.1079x + 0.042 R^2 = 0.9434
Co^{2+}	0.015 ± 0.00702	0.022 ± 0.0032	0.012 ± 0.0095	0.013 ± 0.00439	0.017 ± 0.00565	0.076 ± 0.006136	0.021 ± 0.005005	0.023 ± 0,008127	y = 0,2606x + 0,0028 R^2 = 0,9994
NH_4^+	5.660 ± 1.9769	38.891 ± 2.79303	14.278 ± 1,040658	33.622 ± 4.1827	18.317 ± 3.70221	30.735 ± 4.77235	75.464 ± 3.51938	51.887 ± 4.29301	y = 1402x + 770.9 R^2 = 0.999
Inorganic anions (mg/L)									Concentration calibration in the range of 0.5–60 mg/L
F^-	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	LOD 0.628 μg/L LOQ 200 μg/L
Cl^-	50.96 ± 3.29	54.33 ± 3.33	50 ± 2.67	36.72 ± 7.08	25.25 ± 1.33	32.51 ± 0.493	40.76 ± 1.275	45.87 ± 1.403	y = 257638x − 38436 R^2 = 0.9932 LOD 2.667 μg/L LOQ 500 μg/L
NO_2^-	nd	nd	nd	nd	nd	nd	nd	Nd	LOD 1.103 μg/L LOQ 200 μg/L
Br^-	17.3 ± 0.83	30.6 ± 0.65	41.4 ± 0.44	13 ± 0.87	12.8 ± 0.32	11.8 ± 0.12	14.3 ± 0.22	10.6 ± 0.24	y = 111447x − 129051 R^2 = 0.998 LOD 1.667 μg/L LOQ 500 μg/L
NO_3^-	8.5 ± 0.1	36.1 ± 0.23	21.7 ± 0.22	1.93 ± 0.15	9.54 ± 0.02	15.9 ± 0.23	13.3 ± 0.11	22.7 ± 0.56	y = 14266x − 134116 R^2 = 0.9998 LOD 1.4815 μg/L LOQ 500 μg/L
SO_4^{2-}	40.7 ± 9.05	53.3 ± 2.08	21.9 ± 3.10	20.9 ± 7.93	34.9 ± 8.21	23.6 ± 7.97	40.3 ± 9.21	20.6 ± 4.97	y = 182013x − 159736 R^2 = 0.9985 LOD 0.640 μg/L LOQ 200 μg/L
HPO_4^{2-}	639 ± 3.25	607 ± 3.00	507 ± 5.83	725 ± 7.17	463 ± 5.23	551 ± 2.67	841 ± 5.32	1150 ± 3.81	y = 84165x − 436940 R^2 = 0.9988 LOD 0.625 μg/L LOQ 500 μg/L

The samples were not manipulated (no sample preparation, no extraction, no filtration, no enrichment) in our study. The matrix has effect on the detection sensitivity. LODs were analysed in pure solvents giving low background noise and high sensitive signals of analytes. When the red wine samples with high ionic strengths were determined, the signal-to-noise 3 LOD was not enough to monitor the peaks. Therefore, the samples were analysed with addition of the standards into the native samples. When then the S/N was 3, the concentration value was accepted as the LOQ. If the LOQ would be calculated according to IUPAC, the results were wrong because the peaks could not be reliable detected.

* Cold maceration.

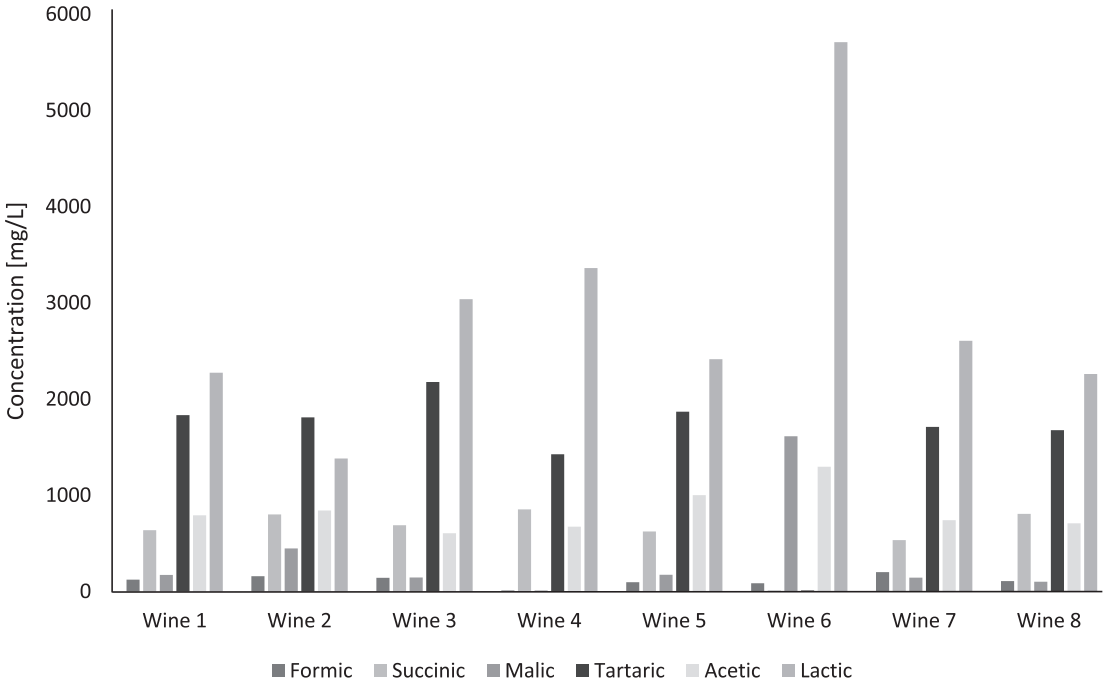

Fig. 1. Carboxylic acids found in the eight *Pinot Noir* red wines. Compounds in order: formate, succinate, malate from malic acid, tartrate, acetate, and lactate. The anions of citric acid and 2-furoic acid were also observed, but they were not quantified. Winemaking processes: Wine 1 – natural fermentation, Wine 2 – natural MLF, Wine 3 – biodynamic fermentation (biodynamic = wine produced from organically grown grapes), Wine 4 – natural MLF, Wine 5 – micro-oxygenation, Wine 6 – yeast, Wine 7 – wild fermentation, Wine 8 – cold maceration. Determination with capillary electrophoresis. Details in experimental. The determinations were made with a P/ACE MDQ capillary electrophoresis instrument equipped with a PDA detector. Conditions: electrolyte 20 mM 2,3-PyDC - 30 mM tricine - 2 mM $Ba(OH)_2$ - 0.5 mM CTAB - 2 M urea (pH 8.06); separation voltage −20 kV; temperature 25 °C; injection with 0.5 psi vacuum for 5 s.

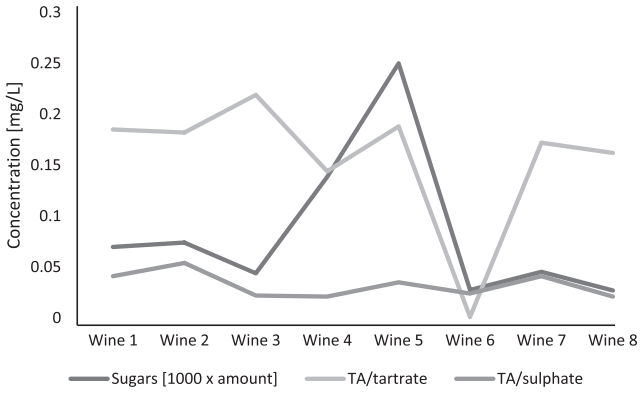

Fig. 2A. Calculation of total acidity (ratio) and sweetness (based on total sugar concentration in the wine) of the *Pinot Noir* red wines. Winemaking processes are described in Table 1.

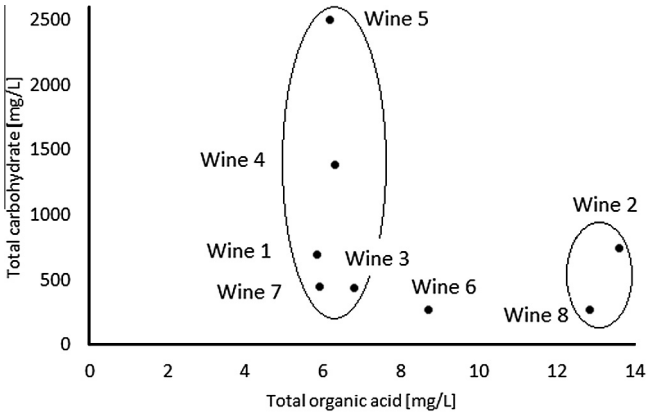

Fig. 2B. Classification of the *Pinot Noir* red wines by their carbohydrates and acids quantities. Winemaking processes are described in Table 1.

absorbances were more intensive for organic wines than for normal wines. On the contrary, the absorbance at 520 nm was lower for organic red wines than normal wines. In that respect, the results showed that Wine 5 (MOX) and Wine 6 (yeast fermentation) have the lowest concentrations of anthocyanins. Based on the high sensitivities at the measured wavelengths the wines also contained higher concentrations of caffeic acid (276 nm) and gallic acid (320 nm) [7,38,40,41]. According to the calculations they also contained a lot more organic material and therefore they were categorized as organic wines. In the literature, anthocyanin content is informed to be 632 mg/kg in *Pinot Noire* grapes [7,42].

All winemakers add sulphites into the wine juice. In the present project we found water soluble sulphates from sulphur dioxide in the wines fermentation solution. In addition, in the presence of SO_2 there were acetaldehyde more than in processes without SO_2 treatment. Several factors are certified to affect the production of

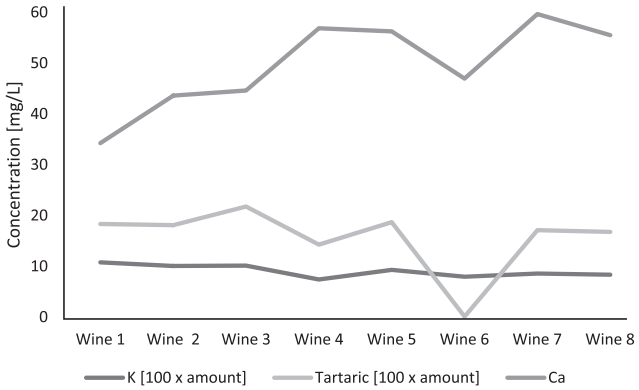

Fig. 3. Correlation of potassium, calcium, and tartrate in the wines. Analyses made with ICP-AES and IC.

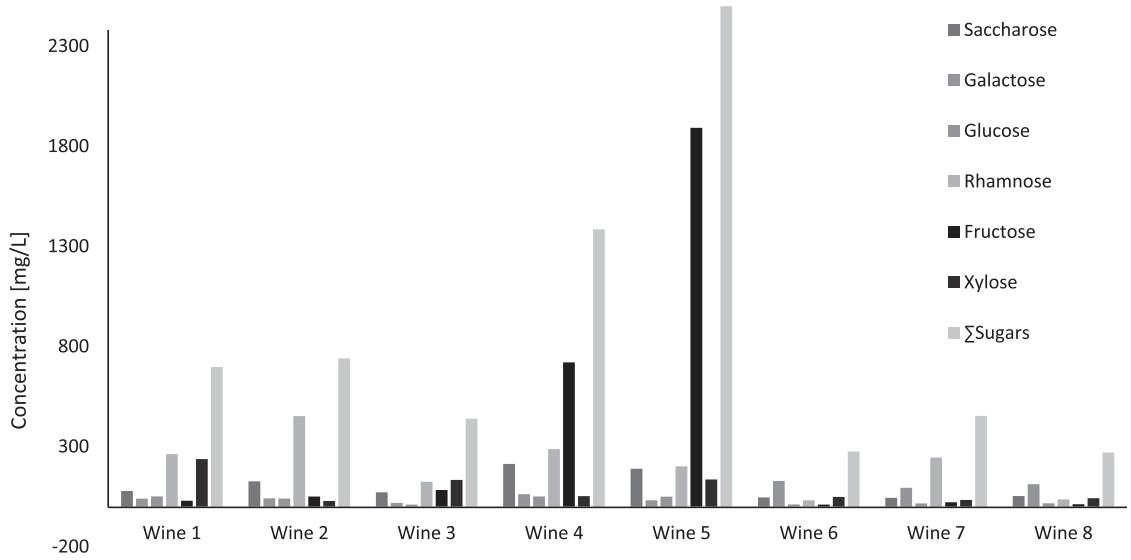

Fig. 4. Carbohydrates in the *Pinot Noir* red wines. Distribution of the carbohydrates compounds in the bottled wines. Compounds in order: saccharose, galactose, glucose, rhamnose, fructose, and xylose. Winemaking process as in Fig. 1. Determination with capillary electrophoresis. The determinations were made with a P/ACE MDQ capillary electrophoresis instrument equipped with a PDA detector. Conditions: electrolyte 130 mM NaOH – 36 mM Na_2HPO_4 (pH 12.6]; separation voltage 20 kV; temperature 20 °C; injection with 0.5 psi vacuum for 5 s.

aldehydes, as yeast strain, temperature, pH, O_2, SO_2, and nutrients. SO_2 affects the enzyme that converts acetaldehyde into ethanol or by complex formation prevents the process [7].

Our study shows that oxygen and SO_2 have impacts on acetaldehyde formation. Because SO_2 is used to prevent oxidation of wine and to decrease growth of LAB, but also to reduce kinetics of enzymatic fermentation, it is added into sweet wines rather more than into dry wines [34,35]. It also stabilizes the pH and forms complexes with aldehydes. In the present study SO_2 was calculated from the experimental results as informed in the references [2,7,8]. The results showed that the quantities were according to regulations, which suggest the limit of SO_2 is 160 mg/L (Table 2).

3.5. Inorganic compounds and metals in the wines

Quantity of inorganic compounds, elements, and metals in wine depend on several factors, such as soil characteristics, type of grape, area of production and environmental conditions [43]. Especially, phosphate (tot-P) in wines is usually related to levels in their musts, but part of it is also from soil [33]. As to sulphate, its main source is sulphite, which is added to wines [44]. According to our results, the concentrations of inorganic compounds were moderately low (Fig. 5A), except that of tot-P, which was the highest in all Wine 5. The conclusion of the quantities of phosphorous and sulphur are approximately 8 and 5 times higher than that of nitrogen, respectively (Table 2). In turn, tot-P in the wines was 1.5 times more than tot-S.

Ammonium was also quantified in the wines. As informed in literature, probably ammonium was used in alcoholic-fermentation to lower the pH of the grape fluids [45]. In addition, bicarbonate (HCO_3) was recognized, which is known to use in chemicals in wine manufacturing. Because potassium was also high, potassium has been added as bicarbonate to *Pinot Noir* wines in order to lower the wine acidity. Assumingly, naturally bicarbonate can also come from water soluble CO_2 produced during fermentable maceration or origin from soil by mineral migration. It is interesting that the biodynamic processed wines without usage of SO_2 and the wine made by MOX produced more soluble CO_2 than the others, which was noticed from the slightly increased pH (Table 2).

The most abundant mineral in the studied wines was potassium. However, according to literature the total mineral elements in wines of different fruit sources may be different [44] and the nutrient elements exist in ionic forms K^+, Ca^{2+}, and Mg^{2+} [7,19,20,21,40,41]. Geographical features have high influence on metal levels of Na, Ca, K, and Fe in grapes (Fig. 5B). Our results

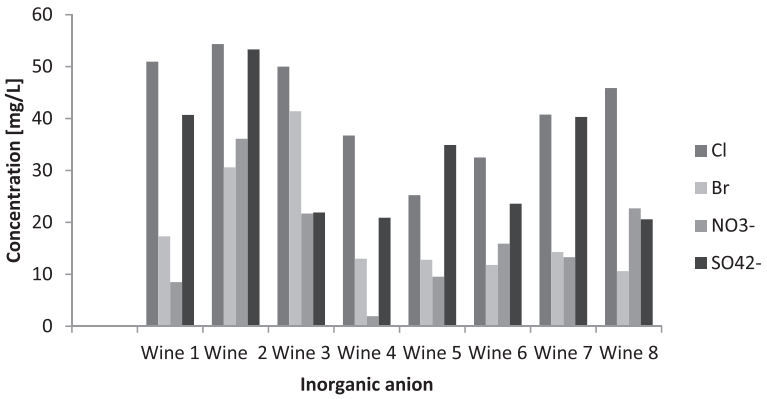

Fig. 5A. Profiles of inorganic anions in the *Pinot Noir* red wines. Winemaking process as in Fig. 1. Determination with capillary electrophoresis. Details in experimental. Analyses made with IC.

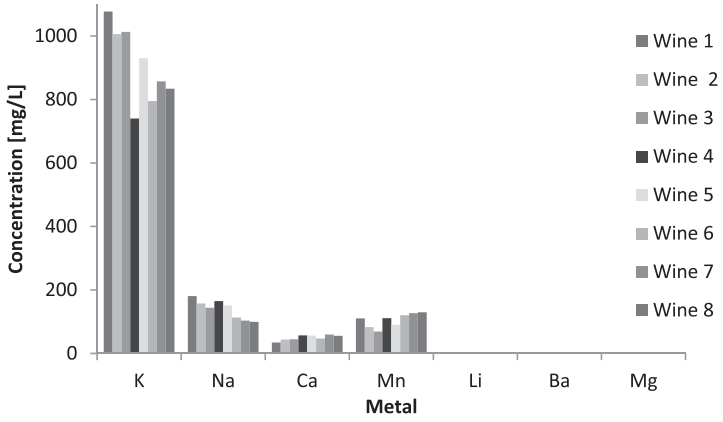

Fig. 5B. Profiles of alkaline and earth alkaline metals in the *Pinot Noir* red wines. Barium, lithium, and magnesium concentrations cannot be seen due to their low values (0.4, 0.09, and 2.1 mg/L, respectively). Determination with ICP-AES. Determination with ICP-AES. Elements with the specific wavelengths were K 766.491 nm; Na 589.592{57} nm; Ca 393.366{85} nm; Mn 257.610{130} nm; Li 670.784{50} nm; Ba 455.403{74} nm; Mg 279.553{120} nm.

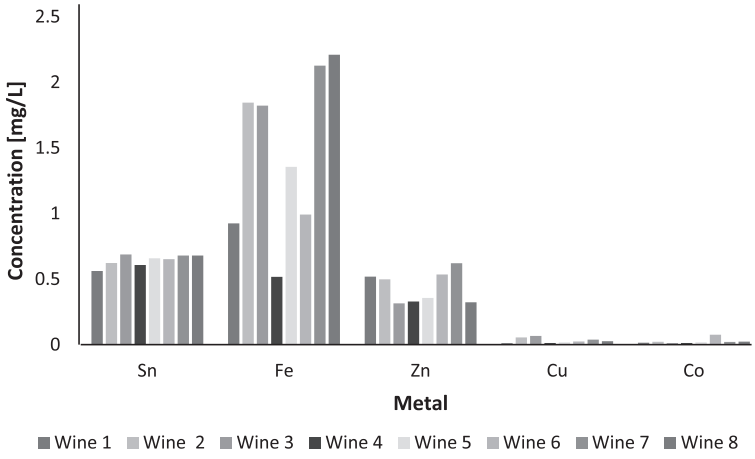

Fig. 5C. The major metal compositions in the *Pinot Noir* red wines. Winemaking process as explained in Fig. 1. Elements with the specific wavelengths were Sn 189.989{176} nm; Fe 239.562{140} nm; Zn 213.856{157} nm; Cu 324.754{103} nm; Co 228.616{147} and 237.862{141} nm.

gave potassium between 0.75–1.1 g/L. Although the total mineral concentrations were only 0.25–0.36 g/L, the total potassium was from 1.05 g/L to 1.4 g/L (highest in Wine 1, lowest in Wines 4 and 6, Table 2). Noteworthy is that in the samples sodium was quite high compared with the recommendations (60 mg/L). In addition, the European wine regulation allows maximal 1.0 g/L potassium sulphate [21–23,45]. From the addition of K_2SO_4 salt it was calculated that the ratio of potassium and sulphate in the salt is 0.814, which in that sense is the limit. Wines 1–3 and 5 were different from the others. Wines 1, 2, and 5 were 0. 662 g/L, 0.618, and 0.571 g/L of sulphate, respectively. The Wine 3 processed from biodynamic grapes has overloading of potassium (0.437 g/L, Table 2).

The concentrations of heavy metals correlate with the quantities noticed by Moreno et al. [19]. It is known that copper form complexes with organic acids, especially with citric acid and therefore minimizes the total acidity of the sample [46,47]. As commonly known, copper was used to increase the pH and decrease the feeling the wine products acidic. The experiment with ICP-AES gave the highest concentration for copper in Wine 3, which also had the highest pH (Fig. 5A). The wines did not contain As, Cd, Cr, Ni, Pb, V, or Ti (less than 1 μg/L, LOD). Usually, lead and cadmium concentrations are 40 μg/L and 0.5 μg/L in the wines [46–49]. However, in the present study, heavy metals Sn, Fe, Zn, Cu, and Co were quite high at level of 0.65 mg/L (highest in Wine 3, smallest in Wine 1), 2.1 mg/L (highest in Wine 8, smallest in Wine 4), 0.6 mg/L (highest in Wine 7, smallest in Wines 3 and 8), 0.068 mg/L (highest in Wine 3, smallest in Wines 1 and 4), and 0.075 mg/L (highest in Wine 6, smallest in Wine 3), respectively.

4. Conclusions

The study showed that the profiles of *Pinot Noir* wines vary with composition of organic and inorganic compounds. Different analytical separation techniques and inorganic and organic analyses were used to obtain the multilateral analytical evaluation. The *Pinot Noire* red wines that were produced in normal winemaking contained higher concentrations of organics than those made with new process technologies. Micro-oxygenation seemed to reduce the quantities of anthocyanins.

Conflict of Interest

No conflict of interest exists in this study.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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